Antibodies against antibodies: immunogenicity of adalimumab as a model
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Citation for published version (APA):
Adalimumab elicits a restricted anti-idiotypic antibody response in autoimmune patients resulting in functional neutralization


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ABSTRACT

Worldwide millions of patients are treated with therapeutic monoclonal antibodies. These biological therapeutics can be immunogenic, resulting in anti drug antibody (ADA) formation which leads to loss of response. Fully human biologics such as the anti-TNF antibody adalimumab are considered to be weakly immunogenic, but we recently detected anti-drug antibodies (ADA) in more than half of the treated rheumatoid arthritis (RA) patients within 28 weeks of treatment. Here we unravelled the mechanism by which ADA lead to loss of response by investigating the full specificity of the repertoire of anti-adalimumab antibodies. The specificity of the repertoire of anti-adalimumab antibodies in a cohort of 50 ADA-positive RA patients was elucidated. Therefore, inhibition experiments using TNF and patient derived anti-adalimumab monoclonal antibodies were performed. The antibody response against adalimumab is highly restricted: Fab fragments of a single, monoclonal antibody specific for the idiotype of adalimumab inhibited 98.65% (25\textsuperscript{th}-75\textsuperscript{th} percentiles: 98.25- 99.90) of the total anti-adalimumab reactivity in sera of 50 different ADA-positive RA patients. Furthermore, we found that the anti-adalimumab response is confined to the TNF binding region of adalimumab, thereby neutralizing its therapeutic efficacy. In line with this restricted specificity, we observed small immune complexes in the circulation of ADA-forming patients. The humoral immune response against adalimumab is highly restricted and limited to the idiotype of the therapeutic antibody. All antibodies result in functional neutralization of the drug, thereby providing a mechanism in which ADA formation leads to clinical non-response.
INTRODUCTION

The use of therapeutic monoclonal antibodies has revolutionized the treatment of many diseases. In recent years, millions of patients have been successfully treated with these biological agents. However, long term treatment with therapeutic monoclonal antibodies can induce anti drug antibody (ADA) formation that is associated with lower drug levels and clinical non-response.\textsuperscript{1-4} The mechanism in which the formation ADA hampers clinical response is still unknown. We hypothesize two possible mechanisms. First, administration of a drug to patients producing ADA leads to the formation of immune complexes which might result in accelerating clearance of the drug. Second, neutralizing ADAs might block the binding of the drug to its target.\textsuperscript{5}

The TNF blocking therapeutic antibody adalimumab is widely used in different inflammatory diseases, such as Rheumatoid Arthritis (RA), Ankylosing Spondylitis and Crohn’s disease.\textsuperscript{6-8} Part of the patients which are chronically treated with adalimumab generate antibody responses against this therapeutic monoclonal antibody which are linked to low functional drug levels and reduced clinical response.\textsuperscript{3,9-14} The percentage of patients developing ADA is underestimated in these studies since in all standard assays the measurement of ADA is hampered by the presence of the drug itself. Recently, we developed a novel assay enabling measurement of anti-adalimumab antibodies in the presence of drug.\textsuperscript{15} Using this assay we have showed that more then half of the adalimumab-treated RA patients produce ADA in the first 28 weeks of treatment. In most of these patients however this does not lead to clinical non-response, since only 22% of patients fail treatment at this time point. To obtain a better insight in the mechanisms by which ADA formation influences treatment efficacy and safety, more knowledge is required on the specificity and the immunological consequences of ADA against adalimumab.

According to the anti-idiotype theory presented by Niels Jerne in the 1970’s the variable region of a given antibody contains several distinct sites (idiotopes) that together form the idiotype, against which a variety of anti-idiotypic antibody molecules can be formed.\textsuperscript{16,17} Idiotopes may be located at the actual antigen binding site of the recognized antibody, and may also include variable region sequences outside of the antigen binding site. Depending on the idiotopes recognized, anti-idiotype antibodies may or may not block the interaction of the antibody with its target antigen.

In this study we unravel the mechanism by which the ADA response against adalimumab leads to clinical non-response. We do so by studying the diversity of epitopes involved in the antibody response against adalimumab and to which extent these are located on the antigen binding part of adalimumab, thus blocking binding to TNF.
MATERIALS AND METHODS

Patients
For the isolation of B-cells, citrated blood was collected from an adalimumab (Abbott, Ilinois, USA) treated patient with Ankylosing Spondylitis and known high titers of ADA. This patient responded very well at the strat of adalimumab treatment but shows increased disease activity after two months. After a total of five months of treatment adalimumab therapy was discontinued. The patient did not suffer from any adverse events. For this study, blood, containing 2690 AE of ADA and no adalimumab, was withdrawn two weeks after ceasing therapy. The study was approved by the local ethics committee.10

For the inhibition studies, some sera of patients from the observational cohort of adalimumab-treated ADA RA patients were used, but mainly adalimumab treated patients send in to our diagnostics department.3;9 Of these patients send in to our diagnostic department no further clinical data are available.

Bridging ELISA for the measurement of ADA
Measurements of ADA in the bridging ELISA were essentially carried out as described before.18 Maxisorp ELISA plates (NUNC) were coated overnight with 100 µl 0.5 µg/ml adalimumab in PBS. After washing 5 times with PBS-Tween (PT), plates were incubated with samples of interest (diluted appropriately in HPE, Sanquin, Amsterdam, the Netherlands). Subsequently the plates were washed and incubated for 1 hour with biotinylated adalimumab (5 ng/ml in HPE). After washing streptavidin-poly-HRP (Sanquin) (1/10,000 in High Performance ELISA buffer (HPE)) was added for 20 minutes for detection. After washing with PT the ELISA was developed with 100 µg/ml tetramethylbenzidine (TMB) in 0.11 M sodium acetate (pH 5.5) containing 0.003% (v/v) H2O2. The reaction was stopped with 2M H2SO4. Absorption at 450 nm was measured with a ELISA reader (Multiskan; TiterTek, Elfab Oy, Finland). Results were related to a titration curve of adalimumab in each plate. The lowest level of detection was 0.001 mg/l. To demonstrate the specificity a similar ELISA was performed using infliximab (Remicade®) immobilized on microtitter plates and biotinylated infliximab as conjugate. For the inhibition experiments using Fab fragments of anti-adalimumab 1.2 the test was essentially carried out as described above with slight modifications. Here, all incubation steps were performed in PTG buffer (PBS, 0.2% gelatin, 0.02% Tween 20) and the conjugate was pre-incubated for two hours with Fab fragments of anti-adalimumab 1.2.

Isolation, proliferation and identification of adalimumab specific single B-cells
Peripheral blood mononuclear cells (PBMC’s) were isolated from citrated blood using a Percoll gradient (Amersham Pharmacia Biotech, Uppsala, Sweden). B-cells were
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Production of recombinant human antibodies
RNA was isolated from wells containing antigen specific B-cells using Trizol (Peqlab, Erlangen, Germany). cDNA synthesis and RACE PCR were performed using the Clontech SMART cDNA synthesis kit (Clontech, Mountain view, USA, cat 634914). RACE PCR products for VL and VH were treated with T4 DNA polymerase and cloned into pcDNA3.3 (Invitrogen) expression vectors containing ligation-independent cloning sites followed by the constant domains of the human Kappa and human IgG1 allotype f genes, essentially as described before. Expression vectors were used for transient transfection of HEK293F cells with 293fectin and OptiMEM (Invitrogen), using the Freestyle HEK293F expression system (Invitrogen) according to the instructions supplied by the manufacturer.

Competition ELISA with recombinant monoclonal antibodies
Maxisorp ELISA plates were coated o/n with 0.5 μg/ml adalimumab in PBS. The binding of biotinylated anti-adalimumab 1.1 and anti-adalimumab 1.2 was analysed after pre-incubation of the coat with increasing amounts of unlabeled anti-adalimumab 1.1 and anti-adalimumab 1.2. All incubations were performed in HPE. After washing streptavidin-poly-HRP (Sanquin) (1:10.000 in HPE) was added for twenty minutes, after which the ELISA was developed using TMB as described above.

Antigen binding test, inhibited by human monoclonal antibodies or rTNF
F(ab’)2 and Fab fragments were generated as described before. The antigen-binding
test (ABT) was described before. One micro liter of serum diluted in PBS/0.3% bovine serum albumin (BSA) (PA buffer) was incubated o/n with 1 mg Sepharose-immobilized protein A (GE healthcare, Chalfont St. Giles, UK) in a final volume of 800 µl. Subsequently the samples were washed with PBS 0.005% Tween and specific ADA binding was detected by o/n incubation with 20,000 dpm (approximately 1 ng) \(^{125}\text{I}\) labeled F(ab')\(_2\) adalimumab diluted in Freeze buffer (Sanquin). Unbound label was removed by washing, and protein A bound radioactivity was measured. Binding of patient antibodies to \(^{125}\text{I}\)-labeled adalimumab F(ab')\(_2\) was inhibited by pre-incubation of the label with increasing amounts of Fab fragments of recombinant anti-adalimumab 1.2 or with rTNF (Strathmann, Hannover, Germany).

**TNF bioassay**

10^4 TNF responsive endothelial cells (ECRF-24 cells) were seeded in a 96 wells plate in IMDM containing 5% FCS (Bodinco), 100 U/ml penicillin, 100 µg/ml streptomycin, 50 µM 2-ME, 20 µg/ml human apo-transferrin (Sigma-Aldrich). After 24 hours of incubation the samples containing TNF, adalimumab and Fab fragments of monoclonal antibody were added. Supernatants were harvested after 24 hrs incubation and tested for IL-8 concentration by ELISA.

**Sucrose gradients**

Sera were analysed by isokinetic sucrose gradient centrifugation. Sucrose gradients (5-32.9%, w/v, sucrose; Merck) were prepared in PBS, pH 7.4 containing 5 mM EDTA and 0.1% Tween (PET). 100µl serum or 500 AE of patient ADA was diluted in 200 µl PET and layered onto the sucrose gradient. The gradients were centrifuged in a Beckman swing out rotor type SW 41 at 36,000 rpm for 16 hours at 20°C. Fractions of 500 µl were collected and ADA levels were detected using the ABT and the PIA. IgG levels were determined by ELISA. Briefly, Maxisorp ELISA plates were coated o/n with 1µg/ml Sheep-anti-human IgG (SH16-1) (Sanquin) in PBS. For detection 1 µg/ml biotinylated monoclonal anti human IgG (MH16-1) (Sanquin) in HPE was used. Afterwards MH16-1 binding was detected using poly-HRP and TMB as described above.

**RESULTS**

**Isolation and characterization of anti-adalimumab producing B-cells.**

In order to investigate the mechanism by which ADA lead to clinical non-response we first wanted to study the number of epitopes involved in the immune response against adalimumab. Therefore we obtained adalimumab-specific monoclonal antibodies.
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Figure 1: Anti-adalimumab 1.1 and anti-adalimumab 1.2 compete for binding to adalimumab A) The detection of increasing amounts of anti-adalimumab 1.1 and anti-adalimumab 1.2 in a bridging ELISA for the measurement of anti-adalimumab and a bridging ELISA for the measurement of anti-infliximab. B) Increasing amounts of anti-adalimumab 1.2 Fab inhibit binding of adalimumab specific ADA in patient serum but not infliximab specific ADA tested in the ABT. C) Titration of biotinylated anti-adalimumab 1.1 and anti-adalimumab 1.2 in the absence or presence of 12.5 µg/ml unlabeled anti-adalimumab 1.1 or 12.5 µg/ml unlabeled anti-adalimumab 1.2. D) Inhibition of biotinylated anti-adalimumab by non-biotinylated anti-adalimumab is dose-dependent.
Using these antibodies as a tool in subsequent inhibition studies we aimed at mapping the anti-adalimumab response. To obtain anti-adalimumab antibodies, adalimumab-specific B cells were isolated from an adalimumab-treated patient were sorted by flow cytometry. Six positive clones (as tested my measuring supernatant in the bridging ELISA were obtained from the CD27+ B-cell population, from which RNA was extracted (see table S1). Two antibodies were recombinantly expressed leading to two IgG1 monoclonal antibodies designated anti-adalimumab 1.1 (originally identified as an IgG1 antibody) and anti-adalimumab 1.2 (originally identified as an IgG4 antibody). Both monoclonal antibodies underwent extensive somatic hypermutation and originated from different precursor B-cells (see table S2). Both monoclonal antibodies specifically bound to adalimumab and neither showed cross-reactivity with infliximab (another therapeutic anti-TNF monoclonal antibody) (figure 1A).

The antibody response to adalimumab in RA patients is restricted to the idiotype
We investigated how representative these two antibodies are for the total antibody repertoire found in patients sera. First, an inhibition study was carried out with serum

![Figure 2: Binding of serum ADA of fifty patients to adalimumab is inhibited by anti-adalimumab 1.2. A) Ten patient sera containing ADA against adalimumab were inhibited by the addition of increasing amounts of anti-adalimumab 1.2 Fab in the ABT. Results were normalized to the binding percentage of the non-inhibited sample. Binding was significantly (p<0.0001) inhibited by the addition of 2 µg Fab fragments (paired t-test). B) Five representative patients sera containing ADA are inhibited by Fab fragments of anti-adalimumab 1.2 as tested in the bridging ELISA. Again binding was significantly (p<0.0001) inhibited by the addition of 2 µg Fab fragments (paired t-test). C) The percentage inhibition in the total of 50 patients tested in either the ELISA or ABT (mean and standard deviation: 98.52 ± 0.99).]
Adalimumab elicits an anti-idiotypic antibody response resulting in functional neutralization from the patient that served as B cell donor for the generation of recombinant antibodies. Remarkably, in an antigen binding test (ABT) specific for anti-idiotype antibodies, Fab fragments of anti-adalimumab 1.2 prevented 99% of the adalimumab specific reactivity from binding to radiolabeled adalimumab F(ab’)2 (figure 1B), showing that the total antibody response in this patient is directed against the same region as anti-adalimumab 1.2. In line with this finding, binding of either biotinylated anti-adalimumab 1.1 or 1.2 to adalimumab could be dose-dependently inhibited by unlabeled anti-adalimumab 1.1, as well as anti-adalimumab 1.2 (figure 1C/D), indicating that both antibodies bind to overlapping epitopes on adalimumab. In contrast, binding of anti-infliximab antibodies to infliximab F(ab’)2 was not influenced by addition of anti-adalimumab 1.2 Fab fragments (figure 1B). Next, nine other anti-adalimumab positive patients were tested in the same assay (figure 2A). In all cases, the antibody response to adalimumab could be inhibited for at least 98% by anti-adalimumab 1.2 Fab. This indicates that a single monoclonal Fab fragment is able to inhibit all anti-idiotype antibodies present in patient sera, suggesting that the entire ADA response in these patients is directed against the same or overlapping epitopes of adalimumab.

In a larger group of 40 different patients, a similar inhibition assay was carried out using a bridging ELISA that also allows detection of antibodies against the constant region of adalimumab. Pre-incubation of the conjugate with Fab fragments of anti-adalimumab 1.2 again inhibited the signal 94-99% (figure 2B), while pre-incubation with irrelevant human IgG Fab fragments had no effect (data not shown). Thus, the antibody response in sera of in total fifty anti-adalimumab positive patients tested either in the ABT (10 patients) or the bridging ELISA (40 patients), could be inhibited for 94-99% (median: 98.65%; 25th-75th percentiles: 98.25- 99.90) (figure 2C) by a single monoclonal antibody (anti-

Figure 3: The binding of adalimumab to TNF can be neutralized by anti-adalimumab 1.2. In response to 1 ng of TNF ECRF-24 cells produce IL-8 in the linear range of the titration curve. Both adalimumab and infliximab neutralize TNF, thereby preventing IL-8 production (p<0.0001 (adalimumab); p=0.0001 (infliximab). Anti-adalimumab 1.2 rescues IL-8 production in the presence of adalimumab (p<0.0001), but not infliximab (n=3). Results represent mean and SEM (error bars). P values are calculated using an unpaired t-test.
adalimumab 1.2). This demonstrates that the antibody response against adalimumab is highly restricted. The vast majority of the antibodies bind to overlapping epitopes.

All antibodies against adalimumab are neutralizing
We next investigated whether anti-adalimumab 1.2 was directed against the TNF-binding region of adalimumab using a TNF bio-assay (figure 3). In response to TNF ECRF-24 cells produce IL-8 which, as expected, can be inhibited by addition of adalimumab or infliximab (figure 3). Addition of an excess of anti-adalimumab 1.2 Fab fragments prevented inhibition of TNF-induced IL-8 production by adalimumab showing that anti-adalimumab 1.2 neutralizes the effect of adalimumab, while the inhibitory effect of infliximab was not affected (figure 3). In the absence of adalimumab, the recombinant monoclonal antibody had no agonistic or antagonistic effect on the ECRF-24 cells (see figure S1).

All tested anti-adalimumab positive patient sera were able to neutralize adalimumab activity in the TNF bioassay, showing that in all patients at least part of the ADA are neutralizing. To investigate which fraction of the polyclonal response is neutralizing we performed an ABT in which radiolabeled adalimumab F(ab’)2 was pre-incubated with increasing amounts of TNF. The presence of ADA-drug complexes in serum may lead to false-positive results due to cross-linking of radiolabeled adalimumab F(ab’)2 to complex-derived adalimumab by the homotrimeric TNF (for cartoon see figure S2). To circumvent this, we fractionated sera of six patients with high anti-adalimumab levels on sucrose gradients to separate the free monomeric ADA from ADA-drug complexes as we described before.\textsuperscript{15} TNF dose-dependently inhibited the binding to radiolabeled adalimumab F(ab’)2 of anti-adalimumab, derived from six patient sera as well as monoclonal anti-
Adalimumab elicits an anti-idiotype antibody response resulting in functional neutralization of adalimumab 1.2 (figure 4). These data clearly show that the humoral immune response against adalimumab in all tested patients is restricted to the TNF binding region of adalimumab.

The restricted immune response against adalimumab leads to small immune complexes. An antibody response against a very restricted immunogenic region, as we describe here for adalimumab, is expected to result in formation of small immune complexes which might not be efficiently cleared from the circulation. We recently described a new assay which is able to measure anti drug antibodies in complex with adalimumab, enabling us to analyze the size of adalimumab/anti-adalimumab complexes in sera of patients. To this end we fractionated ADA positive patient sera with difference levels of ADA and adalimumab, collected just prior to the next administration of adalimumab, on sucrose gradients and measured which fractions contained adalimumab/anti-adalimumab complexes. This revealed the presence of small immune complexes in all fourteen patients tested. The results of three representative patients are shown in figure 5. In some patients adalimumab/anti-adalimumab complexes can be detected next to free uncomplexed anti-adalimumab, while in other patients all anti-adalimumab antibodies are present in small immune complexes. The measurement of marker proteins in the different fractions suggests that these complexes are no larger than IgG dimers. To aim for maximal separation of monomeric IgG and small immune complexes, sucrose gradients were run at 20°C. Under these conditions, larger immune complexes may have end up at the bottom. However, additional experiments using sucrose gradients centrifuged at 4°C did not reveal any larger complexes (data not shown). Thus, the restricted antibody response against adalimumab leads to the formation of small circulating immune complexes.
complexes, which can be detected in patients that receive adalimumab therapy.

**DISCUSSION**

Antibody formation against adalimumab is increasingly being recognized as a major cause of treatment failure. However, until now the mechanism in which ADA effects treatment efficacy is not yet elucidated. We propose two possible mechanisms. First the formation of immune complexes between ADA and therapeutic antibody may lead to increased clearance of the drug, resulting in suboptimal dosing of the drug. Second, the antibodies may be functionally neutralizing, thus directly affecting treatment efficacy. Here we show that in the case of adalimumab virtually all antibodies against adalimumab are neutralizing and that the ADA response is very restricted leading to the formation of small immune complexes. Since small immune complexes are thought to clear slowly suggesting that the effect of ADA formation on treatment efficacy is probably mainly dependent on neutralization of the drug. This implicates that for adalimumab routine measurements of binding antibodies will be sufficient without requirement to test for their neutralizing capacity. In light of these data, it would be very valuable to determine if the antibody response against human therapeutic antibodies is always restricted to the idiotype and to analyze if the immune response against humanized and chimeric monoclonal antibodies is less restricted.

In light of these new data showing that all antibodies are neutralizing, it might seem contradictory that only part of the ADA producing patients lose responsiveness. However, we hypothesise that clinical response is dependent on the magnitude of the immune response. Low ADA levels might not alter clinical response of patients since here only a small portion of the adalimumab is neutralized. Therefore these patients would still have sufficient drug levels to respond to treatment. Only in patients which produce high levels of ADA, the majority or all adalimumab is neutralized leading to non response to treatment.

Yet another mechanism by which ADA could interfere with effectiveness of the therapeutic monoclonal antibody is in case ADA bear an “internal image” of the antigen. Previous studies by others have shown that an antigen bound by a particular antigen combining region (idiotype) of an antibody may be functionally mimicked by a secondary anti-idiotype antibody that binds to the same region of that idiotype. For therapeutic antibodies this holds the risk that anti-idiotype antibodies against the therapeutic antibody may resemble the target protein and in case of adalimumab therefore react with the TNF-receptor. This could be detrimental for the effectiveness of the therapeutic monoclonal antibody. However, the anti-idiotype monoclonal antibodies against adalimumab that
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we isolated and subsequently cloned showed no effect at all on the TNF-receptor as we tested in a TNF bio-assay (figure S1). Reactivity of anti-idiotype antibodies against adalimumab with the TNF receptor can only be expected if adalimumab binds to the exact same region on TNF as the TNF receptor does.

Knowledge of the mechanism by which ADA interfere with drug efficacy can be important for clinical purposes. For instance, the restrictive nature of the response against adalimumab can be explained by either one major immunogenic idiotope in adalimumab or steric hindrance when all antibodies are binding to idiotopes in close proximity to each other on the idiotype of adalimumab. It would be interesting to investigate this further and identify the precise immunogenic B-cell epitopes of adalimumab. Eventually this could lead to the production of less immunogenic variants of adalimumab. Of course alterations might subsequently lead to the introduction of new immunogenic epitopes or loss of function of the therapeutic antibody.

The presence of large amounts of small immune complexes may have clinical consequences. On the one hand, it might be expected that clinical consequences of small immune complexes are limited, because of little complement activation and Fc-gamma receptor triggering. On the other hand, the existence of long lasting small immune complexes may raise the risk of type III hypersensitivity reactions, as occurring in diseases as serum sickness and SLE. A recently published paper shows an increased incidence of severe venous and arterial tromboembolic events in patients that produce ADA against adalimumab. It would be interesting to investigate whether the presence of these immune complexes leads to serious side effects. In this light, our previous finding that most patients make antibodies against adalimumab, even at levels that do not preclude full neutralization, gain new importance. It is likely that in most patients small circulating immune complexes are chronically induced.

In conclusion the following picture emerges. After repeated treatment with adalimumab, the majority of patients develop ADA. The antibodies are hyper mutated and of the IgG1 and IgG4 isotype. The antibody response is very restricted to the idiotype region of adalimumab. All antibodies neutralize adalimumab function and give rise to small immune complexes which can be detected in the circulation of patients that are being treated with the therapeutic antibody. Depending on the magnitude of the immune response the clinical effect might be limited if only a small portion of the adalimumab is neutralized. However, if high levels of ADA are produced all adalimumab is neutralized leading to clinical non-respons.
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ACKNOWLEDGEMENTS

We would like to thank Erik Mul and Floris van Alphen for their help with the FACS sorting, Gestur Vidarsson for sharing his expertise on the different cloning steps and Paul Parren for critically reading the manuscript.
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SUPPLEMENTARY DATA

Figure S1: Neither anti-adalimumab 1.1 nor anti-adalimumab 1.2 have a direct agonistic or antagonistic effect on the TNF receptor. The level of IL-8 secretion was not significantly altered after incubation of ECRF-24 cells with 2 µg/ml anti-adalimumab 1.1 or anti-adalimumab 1.2 in the absence or presence of 1 µg/ml TNF. Results are expressed as percentage IL-8 production as compared to the non-inhibited condition (100%).

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<th>Anti-adalimumab 1.1</th>
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<tr>
<td>1 ng/ml TNFα</td>
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<td>2 µg/ml</td>
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Figure S2: The presence of immune complexes influenced the inhibition experiments using TNF. In the inhibition experiments using TNF the presence of ADA-drug complexes in serum may lead to false positive results due to cross-linking of radiolabeled adalimumab F(ab’)2 to complex-derived adalimumab by the homotrimeric TNF.

Supplementary table I): The CH and CL usage of the different anti-adalimumab producing B-cells. 192 CD27+ B-cells and 192 CD27- B-cells were sorted and seeded as a single cell per well. After 14 days of culture the supernatants of six anti-adalimumab positive wells (all in the CD27+ population) were tested for the presence of kappa or lambda light chains and the presence of adalimumab specific IgG1 or IgG4 antibodies. *Recombinantly expressed as anti-adalimumab 1.1. **Recombinantly expressed as an IgG1 antibody, anti-adalimumab 1.2.
Supplementary table II): Anti-adalimumab 1.1 and anti-adalimumab 1.2 are derived from different precursor cells and are extensively hyper mutated. V(D)J usage of anti-adalimumab 1.1 and anti-adalimumab 1.2, the CDR3 length and the number mutations leading to amino acid replacements (R) or silent mutations (S).

<table>
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<td>V1-02*02</td>
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Chapter 5

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